PATHOZYME DIAGNOSTICS

LIQVIPATH ADENOSINE DEAMINASE (ADA) (ENZYMATIC KINETIC METHOD)

CAT NO.: ADA

Reagent kit for quantitative estimation of Adenosine Deaminase (ADA) activity in Serum, Plasma, Pericardial, Pleuric Liquid & CSF or Ascetic Fluids.

DIAGNOSTIC SIGNIFICANCE:

The Adenosine Deaminase (ADA) is an enzyme of the purine salvage pathway; it catalyzes the hydrolysis of the adenosine to inosine. An increase of ADA activity in pleural fluid is useful in the assessment of tuberculosis pleural effusion.

PRINCIPLE:

The ADA assay is based on the enzymatic deamination of Adenosine to Inosine which is converted to hypoxanthine by Purine Nucleoside Phosphorylase (PNP). Hypoxanthine is then converted to Uric Acid and Hydrogen Peroxide (H_2O_2) by Xanthine Oxidase (XOD) is futher reacted with Chromogen and 4-AAP in the presence of Peroxidase (POD) to generate Quinone Dye which is measured in kinetic manner.

Adenosine + H_2O $\xrightarrow{ADA}$ Inosine + NH_3

Inosine + PI $\xrightarrow{PNP}$ Hypozanthine + Ribose-l-phosphate

Hypozanthine +2 H_2O + 2 O_2 $\xrightarrow{XOD}$ Uric Acid + 2 H_2O_2

2 H_2O +4-AAP+EHSPT $\xrightarrow{POD}$ 2 H_2O + Quinone dye

SPECIMEN COLLECTION:

No haemolyzed fresh Serum, Pleuric Liquid, CSF and Plasma. Venous Blood should be collected and handled anaerobically. Do not use Citrate or Oxalate as anticoagulant. **DO NOT USE haemolyzed samples, because blood cells have very high concentration of ADA.**

KIT PRESENTATION:

Pack Size	1 X 15 ml	1 X 24 ml
R1- ADA (Enzyme Reagent)	1 X 10 ml	1 X 16 ml
R2- ADA (Substrate Reagent)	1 X 5 ml	1 X 8 ml

WORKING REAGENT PREPARATION:

R1-ADA (Enzyme Reagent) and R2-ADA (Substrate Reagent) are ready to use.

REAGENT STORAGE AND STABILITY:

All reagents are stable at 2-8^{0}C until the expiry date stated on the label.

ASSAY PARAMETERS:

Reaction	: Kinetic	Sample Volume	: 10 µl
Wavelength - I	: 546 nm	R1 + R2 Volume	: 360 µl + 180 µl
Wavelength - II	: 630 nm	Factor	: 1743
Flow Cell Temp.	: 37 ^{0}C	Rea. Slop	: Increasing
Initial Delay	: 300 Sec	Zero Setting	: Dist. Water
Interval Time	: 60 Sec	Linearity	: 200 IU/L
Read Time	: 180 Sec	Sensitivity	: 1.0 IU/L
No. of Reading	: 03	Unit	: IU/L

PROCEDURE:

For Serum/Pleuric Liquid/ Plasma / Pericardial / CSF or Ascetic Fluids

Addition Sequence	Test
R1-ADA (Enzyme Reagent)	360 µl
Sample (Test)	10 µl
Mix & incubate for 3 Minutes at 37 ^{0}C and then add	
R2-ADA (Substrate Reagent)	180 µl

Mix immediately and read **first** absorbance of test exactly at 300 seconds and then, **second, third** and **fourth** at an interval of 60 seconds at Primary wavelength 546nm and Secondary wavelength 630nm. Determine the mean change in absorbance per minute (ΔA/min) Test and calculate the test results.

CALCULATION:

ADA Activity in Test (IU/L) = ΔA/min of Test X 1743

NORMAL VALUES OF Serum/ Pleuric Liquid/ Plasma / Pericardial or Ascetic Fluids:

Normal	Suspect MTB	Positive
Up to 43 IU/L	43 - 62 IU/L	More Than 62 IU/L

NORMAL VALUES OF CSF:

Normal	Suspect TBM	Positive
Less Than 11 IU/L	11 – 12.35 IU/L	More Than 12.35 IU/L

It is advisable that every laboratory determine its own normal / reference values.

LINEARITY: This method is linear up to **200 IU/L**. For values above 200 IU/L, dilute the sample suitably with 0.9 % saline, and repeat the assay. Apply correction due to dilution to arrive at a final result.

REFERENCES:

1. Kalkan A, Bult V, Erl O, Avei S. and Binggol N.K. Adenosine Deaminase and Guanosine Deaminase activities in sera of patients with viral hepatitis. Mem Inst. Oswalo Cruz 94(3) 383-386 (1999).

2. Burgess L. J. Matitz F. J. Le Roux I, et al. Use Adenosine Deaminase as a diagnostics tool for Tuberculosis Pleurisy. Thorax 50:672-674 (1995).

IFU No.: 001/00 | *Rev. No.: 00/120723*

Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

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